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Evidence for major histocompatibility complex restriction in transplantation immunity

(skin-specific antigens/histocompatibility-Y antigen/parathyroid transplants)

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ABSTRACT **Studies on the survival of histocompatibility-Y antigen (H-Y)-incompatible and skin-specific antigen (Skn)-incompatible skin grafts in mice, as well as those concerned with the survival of cultured parathyroid allografts in rats, indicate that grafts provoke a strong immune response only if they include donor macrophages (or Langerhans cells) or if major histocompatibility complex-compatible macrophages are available to react with cells bearing the foreign antigens.**

Although a major histocompatibility complex (MHC) restriction with respect to transplantation antigens has been amply and repeatedly demonstrated *in vitro* (1–7), there is no direct evidence that it plays a role in sensitizing hosts to skin and other organ allografts *in vivo* (but see refs. 8–10). We provide evidence for such a role. We propose that for a graft to provoke a strong immune response, it is essential that some of its transplantation antigens be expressed on donor macrophages (or cells of this "family") or be presented to the host by macrophages which are MHC-compatible with the graft.

Our support for this proposition originates from a series of seemingly unrelated experiments: some concerned with the behavior of skin grafts incompatible with respect to male-specific histocompatibility-Y (H-Y) or skin-specific (Skn) antigens in mice, and others concerned with the behavior of cultured endocrine allografts in rats. The details of the rat experiment are being published elsewhere (11), and most of the data concerning H-Y-incompatible grafts originate from a previous study (12). Thus, although what follows will include data from all of these studies, only the experiments involving Skn will be described in detail.

MATERIALS AND METHODS

Male and female A/J (hereafter A; *H-2*^{a}) and C57BL/6J (hereafter B6; *H-2*^{b}) mice and their F_1 hybrids (B6/A) were used. Tolerance was induced by inoculating B6 mice (less than 24 hr old) with 2×10^7 B6/A spleen and lymph node cells shortly after they had been sublethally irradiated (400 rads of ^{137}Cs irradiation at a dose rate of 98 rads/min). The tolerance-inducing inoculum was prepared in Hanks' balanced salt solution as described (13) and was administered in a standard volume of 0.1 ml of medium through the orbital branch of the anterior facial vein.

Neonatal strain A skin grafts, comprising approximately half of the integument of the trunk, were prepared from male or female mice less than 24 hr old. H-Y-incompatible grafts were avoided. Adult grafts (which were of full-thickness body skin) and all original neonatal grafts that were regrafted from one tolerant B6 host to another were 1 cm^2. Skin grafting was carried out in accordance with the procedure fully described by Billingham (14). Bandages were removed on the ninth postoperative day, and grafts were evaluated daily for 30 days and at least twice weekly thereafter.

To verify that each neonatally treated B6 mouse that rejected a strain A skin graft was nevertheless tolerant of strain A transplantation antigens (i.e., other than Skn antigens), each was subsequently challenged in the pinna of the ear with one-third or one-fourth of a neonatal strain A female heart, following procedures described by Jirsch *et al.* (15). The survival of these fragments was confirmed both visually, under a dissecting microscope, and electrocardiographically (Grass Instruments; model 79 D polygraph).

RESULTS AND DISCUSSION

Whereas B6 female mice uniformly reject H-Y-incompatible adult male skin isografts [their median survival time (MST) is about 20 days], neonatal male skin isografts often are accepted (12). Moreover, such newborn grafts frequently render their hosts unresponsive to adult male skin, especially if they precede the latter. Nevertheless, these neonatal grafts remain immunogenic because when they are subsequently removed and transplanted to secondary B6 females, they are rejected almost invariably. It was found that 27 of 30 originally neonatal male skin grafts maintained on normal females for 100–250 days were rejected within 80 days (MST: 32 days) when retransplanted to second female host (ref. 12, unpublished data).

In contrast to these observations are our results with Skn antigens—antigens that behave similarly to H-Y not only in terms of the reactions they provoke but also with regard to their behavior in neonatal skin grafts as well (16). Thus, B6 mice rendered tolerant at birth with B6/A lymphoid cells not only usually rejected adult strain A skin grafts (which, of course, provides evidence for the tissue specificity of the antigens involved) with a MST similar to the MST of H-Y-incompatible grafts in B6 mice (ref. 16; Table 1, experiment 1), but also, as in the H-Y case, these tolerant mice frequently accepted neonatal strain A skin grafts. Furthermore, such grafts, like H-Y-incompatible neonatal skin grafts, often render their hosts tolerant of subsequently placed grafts of adult skin (16). However, this is where the similarity ends for, unlike the situation with H-Y antigen, we observed such Skn-incompatible grafts to be accepted when they were removed from their primary hosts where they had survived for 100–150 days, and were regrafted to secondary hosts which, like the first hosts, also had been made tolerant at birth with B6/A spleen and lymph node cells (Table 1, experiment 2). Moreover, we also can report that the privilege

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Abbreviations: MHC, major histocompatibility complex; MST, median survival time; H-Y antigen, histocompatibility-Y antigen; Skn antigen, skin-specific antigen.

Table 1. Survival of neonatal or adult strain A skin grafts on tolerant B6 hosts after being maintained for various periods on either unresponsive B6 or A/B6 F_1 hybrid mice

Exp.	Source of graft	Primary host	Maintained days	No.	Survival on B6 host,* days
1	Adult	—	—	11	13, 18, 2 × 19, 20, 22, 27, 31, 32, 2 × >100
2	Neonatal	B6	100–150	8	8 × >100
3	Adult	B6	100–150	7	38, 6 × >100†
4	Neonatal	A/B6	100	6	41, 77, 4 ×>100‡
5	Adult	A/B6	150	8	2 × 28, 50, 62, 2 × 64, 65, >100
6	Neonatal	B6	27	5	24, 25, 36, 71, >100§

* Tolerant of A/B6 lymphoid cells

† One of these grafts became progressively smaller and was scored as rejected at 105 days; another persisted at ≈ 50% of its original size.

‡ One of these grafts became progressively smaller and at 100 days was <5% of its original size; another was ≈ 50% of its original size.

§ At 100 days this graft was ≈ 60% of its original size.

afforded these Skn-incompatible grafts is not solely a function of their original neonatal condition. Adult strain A grafts that have been maintained for upwards of 100 days on chimeric B6 mice rendered tolerant of Skn antigens by prior exposure to neonatal strain A transplants also frequently are accepted permanently when retransplanted to B6 mice tolerant of strain A (B6/A) lymphoid cells (Table 1, experiment 3).

While we were pondering the basis for these different results with H-Y- and Skn-incompatible grafts, unexpected results also were forthcoming from an unrelated series of experiments concerned with the capacity of cultured parathyroids to survive in MHC-compatible and -incompatible rats. Much to our surprise, we found that when parathyroidectomized Fischer rats were used as hosts for either freshly excised or cultured Lewis rat (MHC-compatible) or ACI rat (MHC-incompatible) whole parathyroids, culturing improved the survival of only the latter. Whereas 1 of 31 Fischer rats accepted freshly transplanted parathyroid glands from ACI rats for >100 days, 8 of 31 animals accepted for this period glands that had been maintained in culture for 26 days. On the other hand, although 21 of 38 Fischer rats accepted fresh parathyroids from Lewis rats for >100 days, only 10 of 31 hosts that were challenged with cultured glands accepted them indefinitely (11).

It occurred to us that if one assumes that grafts provoke a strong immune response only if they include donor macrophages, or if MHC-compatible macrophages are available to react with cells bearing the foreign antigens, that this might explain our paradoxical findings. Thus, if one supposes that the macrophage (Langerhans cell) population of skin, which has been shown to be derived from bone marrow (17, 18), has a life span of about 3 months (19), then male skin isografts maintained on unresponsive B6 females for upwards of 100 days should possess a female Langerhans cell population. Nevertheless, this should not protect such grafts from being rejected by secondary (normal) B6 female hosts inasmuch as this new Langerhans cell population (and, of course, that of the new host) would still be MHC-compatible with the H-Y antigen-bearing cells.

On the other hand, by using similar reasoning, neonatal (or adult) Skn-incompatible strain A skin grafts, maintained for >100 days on B6 mice rendered tolerant at birth with B6/A lymphoid cells should possess a B6 Langerhans cell population; in addition, if MHC restriction is involved, this population because it is MHC-incompatible with strain A, should interfere with recognition of the foreign Skn antigens of the graft when it is retransplanted to a second B6 host that is likewise tolerant of strain A lymphoid cells.

Similarly, the hypothesis also explains why culturing improves the survival of MHC-incompatible parathyroid glands while having no beneficial influence on MHC-compatible transplants. If one assumes that the major influence of maintaining such glands *in vitro* is to eliminate passenger leukocytes, including macrophages, then only when they are transplanted to MHC-compatible hosts would a population of MHC-compatible macrophages be available to present their foreign antigens to the host. Indeed, as far as we are aware, all studies to date that have demonstrated a prolonged survival of otherwise untreated cultured grafts have involved either MHC-incompatible allografts (20–27) or, even more suprisingly, xenografts (28–30).

Precisely where the MHC restriction occurs remains to be determined. It could occur at the level of antigen recognition—i.e., Langerhans cells may only be able to effectively process foreign antigens if they occur on MHC-compatible cells. Alternatively, and more likely, as suggested by Lafferty (31, 32), the restriction may operate at the effector T-cell level. Thus, when B6 Langerhans cells process the Skn antigens of strain A skin grafts, the effector T cells that are produced by the B6 host may only be able to recognize these antigens in association with self ($H\text{-}2^b$) MHC (and not in association with the MHC ($H\text{-}2^a$) of the strain A graft).

It follows that if Skn-incompatible A strain skin grafts are accepted when regrafted from one tolerant B6 host to another because they no longer possess a Langerhans cell population that is MHC-compatible with the cells bearing Skn antigens, they should not fare as well if initially maintained on B6/A hybrids; in this instance, their Langerhans cell population should be of F_1 origin and, hence, MHC-compatible with strain A. This appears to be the case because two of six such neonatal grafts and seven of eight such adult grafts retransplanted from B6/A hybrids (where they had resided for 100–150 days) to B6 hosts tolerant of B6/A lymphoid cells were rejected (Table 1, experiments 4 and 5). Moreover, two of the neonatal grafts that survived displayed some signs of weakness and became progressively contracted, unlike those originally carried by tolerant B6 hosts, which either retained their original size or became larger following regrafting. Indeed, because the behavior of these grafts contrasted so greatly with those that had been initially maintained on tolerant B6 mice, it was necessary to confirm the tolerant nature of the hosts involved. This was accomplished by demonstrating that all of them permanently accepted strain A neonatal heart fragments placed beneath the pinna of the ear (33).

Further evidence that MHC-compatible Langerhans cells contribute to the rejection of Skn-incompatible skin grafts is

provided by the following observations. When five neonatal strain A skin grafts were retained on their initial tolerant B6 hosts for only 27 days (i.e., a period believed insufficient to permit their Langerhans cell populations to be completely replaced by host cells) and then transferred to second tolerant B6 animals, four were rejected and the lone surviving graft was contracted (Table 1, experiment 6). We also believe it is significant that one of five tolerant B6 mice that had retained a neonatal strain A graft in excellent condition for >100 days (after it had resided for >100 days on a primary tolerant B6 host) rejected this graft (in 53 days) after it was challenged on the other side of its thorax with a fresh adult strain A graft (which survived only 16 days). Moreover, neonatal grafts on two other secondary hosts became progressively and severely contracted, like the two grafts described in experiment 4, after they received fresh adult A strain skin grafts (one of which was rejected in 19 days while the other became progressively smaller). We believe that the complete or partial rejections by these mice resulted from the fact that because the fresh A strain grafts with which they were challenged included normal populations of strain A Langerhans cells, these populations were able to process the foreign A strain Skn antigens (to which they must constantly be exposed) and to present them to their hosts in association with an H-2^a haplotype.

The observation that neither regrafted neonatal or adult strain A skin grafts which were maintained on F_1 hybrids for 100–150 days were rejected as promptly as were normal adult A strain grafts (compare results of experiments 4 and 5 with those of experiment 1) could be due to the fact that the Langerhans cells of F_1 mice are not as effective as strain A Langerhans cells in sensitizing B6 mice to the foreign Skn antigens of strain A. Indeed, certain peculiarities in the *in vitro* response of cells from F_1 hybrid females to H-Y-incompatible parental strain cells are well known (2–4, 34). Moreover, it should also be borne in mind that neonatal skin grafts raised on adult hosts are quite different from normal adult skin grafts in terms of their thickness and in the number of hair follicles per unit area. This, too, could contribute to their prolonged survival.

Finally, it should be noted that inasmuch as in these experiments the tolerance-inducing inoculum was prepared from A/B6 spleens and lymph nodes, one might have expected the stem cells of the spleen to have contributed to the Langerhans cell population of the strain A allograft. However, if they did, it did not seem to have any significant effect.

Whereas all the evidence noted above supports our hypothesis, there is one experiment that so far has yielded negative results, but we believe for good reason. This experiment was based on the assumption that if maintaining strain A skin grafts on tolerant B6 mice for >100 days results in replacing their Langerhans cell population with a B6 population, then not only should such grafts display prolonged or permanent survival when regrafted to other tolerant (of B6/A lymphoid cells) B6 mice, but they likewise might survive longer than expected when transferred to *normal* B6 hosts. However, this has proven not to be the case as four such grafts were acutely rejected. Nevertheless, we remain undaunted by this observation as we believe that the B6/A passenger (chimeric) leukocytes, which these grafts almost certainly possessed (35), could very well have been responsible for sensitizing their hosts against the foreign, including MHC, antigens of the graft.

Although our evidence indicates that allografts provoke a strong immune response only if they include donor macrophages (Langerhans cells), or if MHC-compatible macrophages are available to react with cells bearing the foreign antigen(s), this does not rule out the possiblity that, in the absence of such macrophages, alternative forms of antigen presentation may also sensitize the host. However, if this is the case, these other pathways appear to be less efficient and, at least in the case of antigens such as Skn, ineffective.

There are two recent investigations, both involving the transplantation of the heart, which give further credence to our hypothesis. In one of these studies (36) BN rat hearts were accepted permanently by normal MHC-incompatible WAG rats after they had survived for 5–6 weeks in immunosuppressed [by treatment with BN rat blood or with immunosuppressive drugs (37)] animals of this strain; in the other study (38), normal Lewis or BN rats acutely rejected Lewis/BN F_1 hearts after they had functioned for 17–48 weeks in Lewis or BN hosts rendered unresponsive by previous exposure to Lewis/BN F_1 liver allografts.

We are aware that similar rat experiments conducted with AS and AS/WF F_1 hosts and AS/AUG F_1 kidney allografts have yielded different results (39, 40). Such grafts, after surviving in enhanced AS recipients for 1–3 months either displayed prolonged survival or were accepted indefinitely when retransplanted to AS or AS/WF F_1 secondary hosts. Indeed, these grafts were accepted even when the secondary AS recipients were subsequently challenged with AUG/WF F_1 spleen cells. However, we believe that a different mechanism than the one we are propounding here is responsible for these results. As the authors suggest, in these studies it seems more likely that the unresponsiveness is caused by the ability of the long-surviving kidney allografts to induce in their naive secondary hosts the same state of unresponsiveness observed in the first recipient.

Clearly, if the interpretation of our results is correct its clinical implications are obvious. Currently, all efforts are directed, and with good reason, to matching graft donors and hosts with regard to their MHC. However, if the results reported here are verified, they indicte that if culturing, or other means, become available to eliminate passenger cells from allografts, such grafts might be more likely to survive in MHC *incompatible* hosts.

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